REMARKS

By this amendment, claim 1 has been amended. The application includes claims 1-4.

35 U.S.C. §102 (b) rejection

Claims 1-4 stand rejected under 35 U.S.C. §102 (b) as being anticipated by Cheng et al. Examiner states that Cheng et al. teach a DNA vector construct comprising 1) an IRES; 2) a selection marker; and 3) a GFP marker. Regarding claim 2, Examiner states that Cheng et al. teach stable expression of GFP in transduced TF1 cells. Regarding claim 3, Examiner states that Cheng et al. teaches transfection of stem cells. Finally, with regard to claim 4, Examiner states that Cheng et al. teach a reagent to study biological processes.

Cheng et al. describe the transduction of hematopoietic stem cells with a retroviral vector. The retroviral vector contained a GFP reporter gene, and served as a marker to quantify retrovirus-mediated gene transfer into the cells. The purpose of introducing the GFP reporter was to provide a way to evaluate optimization of retroviral transduction, a process that is notoriously inefficient. Using a retroviral vector containing a reporter GFP gene, the authors were able to directly observe the cells that had been successfully transduced. The retroviral vectors that were employed also included a neo selection marker.

The procedure used for transducing the cells is described in detail on page 1020, beginning with the last paragraph of column 1 in the section entitled "Cell lines, production of retroviral supernatants and transduction protocol". As can be seen, it is a multistep procedure that involves making and transducing packaging cells, collecting and preserving supernatants from the growth of these cells, and finally using the supernatants to transduce the cell of interest. The methodology used by Cheng et al. is not unusual. When retroviral vectors are utilized for the introduction of DNA into a host cell, it is necessary to use packaging and/or vector producer cell lines in order to propagate the vector. After the vector is manipulated to contain the DNA of interest, the packaging and/or vector producer cell lines are transfected with the vector and allowed to grow, and the viral vector replicates within the cells and is subsequently isolated from them. The virus is purified and may then be used to transduce or transfect other cells of choice.

Utilizing viral vectors may provide long-term stability of expression of a gene of interest

because viral vectors tend to integrate into the host chromosome. They may then be passed relatively stably from generation to generation (although integrated, non-native DNA sequences are also known to be excised from chromosomal DNA at a high rate in some cases). In any case, however, *the process of integration is random*. This is a distinct disadvantage when working with living organisms. The genome of any organism is arranged in a manner which has been selected via evolution over millions of generations. While some flexibility exists in that certain rearrangements and mutations may be tolerated by the organism, a random, abrupt introduction of a relatively large insertion into the genetic material of a living cell is obviously a less than ideal methodology. In addition, retroviral vectors such as those employed by Cheng et al. are known to include extraneous retroviral genes that are sometimes known to elicit immune reactions in host organisms.

In contrast, the vector of the present invention is non-viral in nature. This is not by accident. A non-viral system was chosen in order to avoid the pitfalls of retroviral systems.

For example, the method of obtaining the transfected cells of the present invention is much simpler. There is no requirement for maintaining a packaging cell line or for growing up and purifying large quantities of the vector so several steps in the process of obtaining stably transfected cells are saved. This is a large advantage in terms of expense and time.

In addition, the cells themselves are rendered "stably transfected" not due to integration of the gene of interest into the host chromosome. Rather, the gene(s) of interest are maintained extrachromosomally on the vector. Stability is ensured by the novel use of the pIRES1*neo* vector in the instant invention, which simplifies the selection and maintenance of positive transfectants. The GFP gene and the neomycin resistance gene (which allows for selection of transfectants via drug resistance) are transcribed on a single mRNA and yet translated as two separate gene products due to the presence of the internal ribosomal entry site (IRES) located between the two coding regions. The pool of stably transfected GFP positive cells obtained from a transfection with the vector of the instant invention will be homogenous with respect to chromosomal DNA, as will their progeny (i.e. the DNA of the host cell will not have been perturbed by integration of a virus); the only variable will be the absolute number of vector molecules (all of which are

identical) that are contained within the individual cells. This offers a clear advantage both in terms of facility of obtaining stable GFP transfectants, and in terms of controlling variation in the cells.

Otherwise, in order to avoid the pitfalls of using viral vectors, one must resort to

1) using two plasmids with the attendant problems of performing double transfections, or

2) using one plasmid which encodes both the gene of interest and the selection gene separately, which would be a much larger plasmid. Larger plasmids are known to be more difficult to deal with technically (e.g. more difficult to get into the cell, to be retained by the cell, etc.). The relatively small size of pIRES*neo* is thus also an asset.

Finally, retroviral genes which are known to cause allergic reactions in some individuals are absent from the vector of the present invention.

The vector and host cells of the present invention are thus fundamentally different from the cells described by Cheng et al. Claims 1, 2 and 4 have now been amended to recite that the DNA vector construct is <u>non-viral</u> in nature, clearly distinguishing the vector, cells and reagent of the present invention from that described by Cheng et al. Support for this amendment may be found, for example, in the Background section of the application where the use of viral vectors is discussed and criticized as generally unfavorable (page 2, lines 20-page 3, lines 1-2). Those of skill in the art would recognize that the vector of the present invention is non-viral. Claim 3 depends from claim 2, and thus should also be patentable.

In view of the foregoing, reconsideration and withdrawal of this rejection are respectfully requested.

Other amendments. Claim 4 has further been amended to recite "the non-viral DNA <u>*vector*</u> construct" (rather than "the non-viral DNA construct") of claim 1 in order to comply with the language used in claim 1, which recites a "vector construct". Applicant submits that this amendment was undertaken simply to preserve correct antecedent basis and thus does not introduce new matter.

Applicant respectfully requests entry of this amendment.

Double Patenting Rejection

Claims 1-4 stand rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-25 of U.S. Patent No. 6,316,181 (Fillmore et al.).

Applicant herewith files a Terminal Disclaimer in which Applicant agrees to disclaim the terminal part of any patent granted on the above-identified application which would extend beyond the expiration date of U.S. Patent No. 6,316,181.

In view of the foregoing, reconsideration and withdrawal of this rejection are respectfully requested.

Formal Matters and Conclusion

In view of the foregoing, Applicant submits that all rejections have been successfully traversed. The Examiner is respectfully requested to pass the above application to issue at the earliest possible time.

Should the Examiner find the application to be other than in condition for allowance, the Examiner is requested to contact the undersigned at the local telephone number listed below to discuss any other changes deemed necessary in a telephonic or personal interview.

Please charge any underpayment or credit any overpayment of fees to attorney's deposit account # 50-2041.

Respectfully submitted,

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